Integrated Master in Chemical Engineering

Microencapsulation of Resveratrol with applicability in food industry

Master's Thesis

of

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Resumo

O interesse da sociedade por produtos alimentares com compostos bioativos na sua composição, como polifenóis, tem aumentado continuamente. Isto deve-se ao facto de estes compostos terem propriedades benéficas na saúde humana, desempenhando um papel importante na prevenção de muitas doenças como cancro e doenças neurodegenerativas e cardiovasculares. No entanto, estes compostos apresentam algumas limitações como instabilidade, serem facilmente oxidados e sensíveis ao calor e à luz. Estas características limitam a sua aplicação na indústria alimentar. A microencapsulação é uma tecnologia eficiente capaz de ultrapassar estas limitações, uma vez que tem a capacidade de proteger e estabilizar os compostos, evitando a sua degradação e controlando a sua libertação. O resveratrol é um polifenol, com ótimas propriedades, nomeadamente antioxidantes e anti-inflamatórias, capaz de previnir doenças como o cancro e doenças cardiovasculares e neurodegenerativas. Este composto encontra-se na casca das uvas vermelhas, no vinho tinto, nos amendoins e noutros alimentos em quantidades reduzidas. Assim, torna-se importante conseguir introduzir estes compostos em produtos alimentares, em quantidades suficientes, para que estes consigam ter o efeito desejado e de forma protegida, de maneira a manter a sua estabilidade e atividade. Este trabalho consiste na microencapsulação do resveratrol pelo método de *Spray drying*, utilizando goma arábica como agente encapsulante. Foram preparados dois tipos de microcápsulas: microcápsulas constituídas por goma árabica, óleo de coco e resveratrol e microcápsulas constituídas unicamente por goma arábica. O rendimento do processo foi calculado e as microcápsulas obtidas foram caraterizadas de acordo com seu tamanho e forma. Adicionalmente, foram efetuados estudos de libertação controlada usando 10, 15 e 20 % de goma árabica nas soluções de agente encapsulante. Por fim, foi calculada a eficiência de encapsulação e aplicados modelos cinéticos de libertação aos perfis obtidos experimentalmente. Os valores de rendimento do processo obtidos para microcápsulas compostas apenas por goma arábica variam entre 43,5 % a 51,7 %, enquanto que para as micropartículas compostas por goma árabica, óleo de coco e resveratrol, variam entre 16,7 % a 22,7 %. No que se refere ao tamanho médio das partículas este varia entre 6,8 μm e 7,8 μm, em termos de distribuição de volume, para cápsulas produzidas apenas com goma arábica. Cápsulas contendo resveratrol obtiveram um tamanho médio de 8.2 μm. A superfície das mesmas foi avaliada através da técnica de microscopia eletrónica de varrimento (SEM). As microcapsulas apresentam uma forma esférica e irregular, independentemente da concentração de material encapsulante utilizado. Os estudos de libertação controlada foram avaliados pelo método de espectrofotometria UV-Vis. Todos os perfis de liberação obtidos mostram um comportamento semelhante. Foi também concluído que as microcápsulas produzidas são eficazes na proteção do resveratrol. O maior valor de eficiência de encapsulação obtida foi de 87 %, usando a solução com 20 % de agente encapsulante. Finalmente, os perfis de libertação ajustam-se bem ao modelo cinético de Weibull.

Palavras Chave: Goma Arábica, Libertação Controlada, Microencapsulação, Resveratrol, Spray-drying

Abstract

Food products with bioactive compounds, like polyphenols, have increased in popularity due to their benefits to human health. They play an important role in the prevention of many diseases, such as cancer and neurodegenerative and cardiovascular diseases. However, these compounds are usually unstable, easily oxidable and sensitive to heat and light, which limits their application in the food industry. Microencapsulation is an efficient technology that can be used to overcome these limitations by protecting and stabilizing the compounds, preventing their degradation and controlling their release. Resveratrol is a polyphenol with great properties namely antioxidants and anti-inflammatory, capable of preventing diseases such as cancer and cardiovascular and neurodegenerative diseases. It is found naturally in the skin of red grapes, red wine, berries, peanuts and other peanut products in low amount. Considering this, it is important to introduce this compound (concentrated and protected) in food products, in order for it to be consumed in the ideal amount, to reach its potential and take the maximum advantage of it.

The purpose of this work was to microencapsulate resveratrol by spray dryer technology, using arabic gum as the encapsulating agent at different concentrations. Two types of microparticles were prepared: one type consisted of arabic gum, coconut oil and resveratrol and other was only composed by arabic gum. The product yield of the process was calculated and the obtained microcapsules were characterized according to their size and shape. Controlled release studies (using microcapsules prepared with 10% (w/V), 15% (w/V) and 20% (w/V) of arabic gum) were also made. Finally, the entrapment efficiency was calculated and kinetic release models were applied, to the release results. Microparticles composed only by arabic gum got a product yield in the range of 43.5 to 51.7 %, while for microparticles composed of arabic gum, coconut oil and resveratrol, got a product yield range from 16.7 to 22.7 %. Regarding microparticles size, the average size obtained for non-load microparticles ranged between 6.8 µm and 7.8 µm. Load particles got an average size of 8.2 µm. The surface of all microparticles was evaluated using scanning electron microscopy (SEM), showing spherical form and irregular shape, regardless of the concentration of wall material that was used. Controlled release studies were assessed by UV-Vis spectrophotometry using absorbance analysis. All the release profiles presented a similar behavior and it was concluded that the obtained microcapsules are effective in the protection of resveratrol. The highest encapsulation efficiency that was obtained was 87 %, for the microparticles prepared with a solution of arabic gum at 20% (w/V). Lastly, the release profiles fitted well with the Weibull model.

Key words: Arabic Gum, Controlled release, Microencapsulation, Resveratrol, Spray-drying.

Declaração

Eu, Teresa Sofia Sousa Cardoso, declaro, sob compromisso de honra, que este trabalho é original e que todas as contribuições não originais foram devidamente referenciadas com identificação da fonte.

Porto, 3 de Julho de 2017

(Teresa Sofia Sousa Cardoso)

Index

List of Figures

List of Tables

Notation and Glossary

t	Time
c	Concentration
t_0	Lag-time
w/w	Mass fraction
w/V	Mass concentration
M_t	Compound release at time t
M_∞	Total release
s_a	Standard deviation of the slope
s_b	Standard deviation of the intercept
β	Shape parameter
τ_D	Time when 63.2 % of compound has been release
K_k	Korsmeyer constante
n	Release exponent

List of acronyms

SEM	Scanning electron microscopy
EE	Entrapment Efficiency
PY	Product Yield
CMC	Carboxymethylcellulose
MD	Maltodextrin
MG	Mesquite gum
AG	Arabic gum
GA-In	Cross-linked Inulin
HPLC	High performance liquid chromatography
UV	Ultraviolet
ROS	Reactive oxygen species
COX	Ciclo-oxigenase
E2(PGE2)	prostaglandina E2
HSV-1	*Herpes simplex* type 1
HSV-2	*Herpes simplex* type 2
PCL	Poly(D,L-lactic-co-glycolic acid)
PHBV	Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)
PLGA	Poly(lactic-*co*-glycolic acid)
HA	Hyaluronic acid
DPPC	Dipalmitoylphosphatidylcholine
LOD	Limit of Detection
LOQ	Limit of Quantification
RSV	Resveratrol
Abs	Absorbance

1 Introduction

1.1 Project presentation

Nowadays, concern on health has increased among the society. The consumer interest in healthier food, containing bioactive ingredients, creates the demand of a technology that allows the incorporation of compounds with great properties in a practicable way in food products (Dias, Ferreira, & Barreiro, 2015).

Natural compounds, like polyphenols, have attracted great interest in functional food, because of their potential benefits on human health. They have antioxidant properties and play an important role in the prevention of diseases which are associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases (Vladimir-kneževi, Blažekovi, Stefan, & Babac, 2012).

Degenerative diseases can appear when the cells are damaged by free radicals. They are capable of attacking the healthy cells causing the loss of their structure and function. The formation of these free radicals is controlled naturally by antioxidants that stabilize or deactivate them before they attack cells. So when there is no equilibrium between antioxidants and free radicals, a condition of oxidative stress follows (Percival, 1998). Therefore, the consumption of food products that are rich in natural antioxidants can prevent some diseases.

There are food products that already have natural antioxidants in their composition. The skin of red grapes, red wine, and peanuts are sources of resveratrol, a polyphenolic compound that has anti-inflammatory and antioxidant properties and is associated with beneficial health effects. However, this compound is present in food products in low amount, making the desirable effect difficult to obtain (Koga, Becraft, Lee, & Lee, 2015). This way, the addition of bioactive compounds supplements in food has become relevant.

Some limitations of polyphenols, like resveratrol, are the fact that they are easily oxidized and sensitive to heat and light, limiting their application in the food industry (J. Aguiar, Estevinho, & Santos, 2016). This way, it is important to preserve their stability, bioactivity and bioavailability.

Microencapsulation can overcome some of the problems above mentioned. This technology is able to protect sensitive compounds from degradation, stabilize them and increase their bioavailability, preserving their properties (Nedovic, Kalusevic, Manojlovic, Levic, & Bugarski, 2011). Besides this, the main advantage of this method is the ability to control the release of compounds. For all the mentioned reasons this method enables the reformulation of a wide variety of food products, allowing its improvement and giving them better and new properties as bioactive roles in the human body (Berta Nogueiro Estevinho, Rocha, Santos, & Alves, 2013).

This project's main goal was the encapsulation of resveratrol by spray-drying technology, so it can be incorporated in food products, in order to increase the consumption and improve its benefits in human health. Microcapsules' characterization was also an accomplished aim in this work.

1.2 Work contributions

This project consists in the microencapsulation of resveratrol, based on the pre-optimization of similar system in the laboratory. With this, it was possible to compare results between microcapsules with different compounds, but produced in the same conditions. There are already some studies in the literature about microencapsulation of resveratrol, but there are no reports in this field when using arabic gum as the encapsulating agent, thus making this project's contribution a progress in this field.

In order to carry out this project a mini spray-dryer BÜCHI B-290 was used for microcapsules production, the quantification of compound was performed by UV-Vis Spectrophotometry, the size distribution of the particles was evaluated by Coulter LS 230 Particle Size Analyzer and microparticles surface structure was performed by scanning electron microscopy (SEM) at Centro de Materiais da Universidade do Porto (CEMUP).

1.3 Thesis organization

This thesis is organized in 8 main sections.

Section 1, called "Introduction" summarizes the project's background motivation and importance, as well as its organization.

Section 2, called "State of the art" is composed by a theoretical introduction about polyphenolic compounds and microencapsulation fundamentals. Microencapsulation applications and an overall review of resveratrol studies in the literature are also addressed.

Section 3, called "Technical Description" describes the procedures and methods used in the project.

Section 4, called "Results and Discussion" contains the obtained results, as well as their analysis.

Section 5, briefly describes the project's main conclusions.

Section 6, called "Evaluation of the work" contains the accomplished goals, limitations and some future work in order to continue the project.

Section 7 presents all the bibliographic references used in this project.

Appendix section contains some additional information of the project.

2 Context and State of the Art

2.1 Polyphenols

Polyphenols are a class of chemical substances, with a common structure. They are composed of at least one aromatic ring, with one or more phenolic units and are the active substance that is present in many medicinal plants. Due to this, they have a protective action on the human health, since they have antioxidant, anti-inflammatory, antibacterial and antiviral properties (Aliakbarian, Paini, Casazza, & Perego, 2015). Polyphenols are the most abundant micronutrients in human diets and their antioxidant properties are one of the main reasons of interest in these bioactive compounds (Manach C, Scalbert A, Morand C, 2004). This property has been extensively studied, due to the polyphenols' ideal chemical structure for the neutralization of free radicals. However, the health effects depend on the amount of the ingested polyphenols and their bioavailability. Polyphenols can be found in greater concentrations in fruits and beverages such as tea, coffee and red wine, but their bioavailability is a major limitation to their activity. Some studies show that polyphenols are poorly absorbed in the intestine and at the same time, highly metabolized by the organism. This way, metabolites that are found in the blood and target organs may differ from the initial substances regarding its biological activity (Manach C, Scalbert A, Morand C, 2004).

Nowadays, it is known that there are more than 8000 phenolic structures that can be divided into ten different groups, according to their chemical structure (Lima, Vianello, Corrêa, Campos, & Borguini, 2014). The main groups are the flavonoids, the phenolic acids and the stilbenoids (El Gharras, 2009). Flavonoids are the amplest class of polyphenols. Stilbenes fit in a small group of these bioactive compounds, composed by two aromatic rings, where one of them carries two hydroxyl groups in the m-position, while the other one may be substituted by hydroxyl and methoxyl groups in different positions (Vladimir-kneževi et al., 2012). This class of polyphenols is found in low quantities in the human diet (Manach C, Scalbert A, Morand C, 2004).

The main reason for the interest in polyphenols is the recognition of their role in the human health and the prevention of many diseases.

Resveratrol is a polyphenol with a wide variety of beneficial properties that belongs to the stilbenes group. It is found in fruits and beverages, but in low concentrations. For this reason, resveratrol properties usually do not have the desired effect in the consumers (El Gharras, 2009). Therefore, it would be of great interest to concentrate this compound and to control its release in the human body in order to take full advantage of its properties in human health.

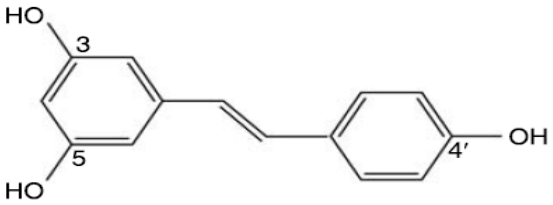
2.2 Resveratrol

Resveratrol (*trans*-resveratrol; *trans*-3,5,4'-trihydroxystilbene), a triphenolic phytoalexin, is a polyphenol from the stilbens family, with a molecular formula of $C_{14}H_{12}O_3$ and molecular weight of 228.25 g mol^{-1} (Aggarwal & Shishodia, 2005; Davidov-Pardo & McClements, 2014). This compound is a white powder, its melting point varies from 253 to 255 ºC and the chemical structure of the molecule consists of two aromatic rings joined by a double bond (Aggarwal & Shishodia, 2005). Concerning its solubility, the compound is highly soluble in ethanol and dimethyl sulfoxide, moderately soluble in triacylglycerol oils and slightly soluble in water (Davidov-Pardo & McClements, 2014). The solubility reported in coconut oil was 0.18 mg mL^{-1} and 0.03 mg mL^{-1}in water (Koga, 2015).

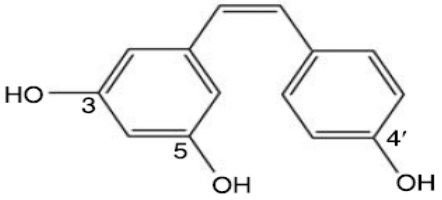
This polyphenol was discovered in 1940 in the roots of white hellebore. It was also found later, in the dried roots of *Polygonum cuspidatum*, called *Ko-jo-kon* in Japanese. These roots were used in traditional Chinese and Japanese medicine (Aggarwal & Shishodia, 2005). In grapevines, it was detected for the first time in 1976 by Langcake and Pryce. It was found that resveratrol is produced in response to fungal infections, pathogenic attacks, environmental stress, UV irradiation, ozone effects, heavy metal ions and changes in climate, in extremely cold conditions, by leaf tissues (Frémont, 2000; Guamán-Balcázar, Setyaningsih, Palma, & Barroso, 2016).

The compound is known to be beneficial for human health and it exists as two isomers, the *cis*-resveratrol and the *trans*-resveratrol (Frémont, 2000). *Trans* and *cis* isomers of resveratrol have a maximum ultraviolet (UV) absorption at 307 and 280 nm, respectively, which allows their detection and separation by high-performance liquid chromatography (HPLC) (Frémont, 2000).

The bioactive form of resveratrol is *trans*-resveratrol, which is the one associated with health benefits and not its isomer, *cis*-resveratrol. *Trans*-resveratrol can turn into the bio-inactive form by the action of light, heat, or pH (Koga, 2015; Koga, Andrade, Ferruzzi, & Lee, 2016). The two isomers of this polyphenolic substance are shown in Figure 1.



Trans-resveratrol (*trans*-3,4′,5-trihydroxystilbene)



Cis-resveratrol (*cis*-3,4′,5-trihydroxystilbene)

Figure 1 – Chemical structure of resveratrol; Extracted from (Aggarwal & Shishodia, 2005).

As it was previously mentioned, resveratrol is sensitive to heat and light. According to the literature, some studies were made, where the effect of heat in resveratrol's degradation was observed. The results showed that after a 20 minutes exposure to 125, 150 and 175 ºC, trans-resveratrol suffered 17, 39 and 70 % degradation, respectively (Zupančič, Lavrič, & Kristl, 2015). In other study performed in blueberries and bilberries, it was shown that after an 18 minutes exposure to 190 ºC, between 17 and 46 % of the resveratrol suffered degradation (Lyons et al., 2003).

Studies to test resveratrol's stability to light were also performed. *Trans*-resveratrol was exposed to UV light at 366 nm during 120 minutes and to sun light during 60 minutes. The results revealed that 90.6 % and 80-90 % of *trans*-resveratrol was converted, in the conditions mentioned before, into *cis*-resveratrol (Koga, 2015). These results are a consequence of resveratrol's photosensitivity.

Resveratrol is naturally present in food, however, another limitation of the compound is the fact that it is located in the seeds and skin of the fruit, so its consumption becomes more limited (Davidov-Pardo & McClements, 2014). Furthermore, it also presents low stability, bioavailability and water solubility (about 0.03 mg mL^{-1}) (Koga, 2015; Zhang, Le, Wang, Zhao, & Chen, 2013). This way, this polyphenol is usually destroyed before it is consumed, since, after been orally consumed, the compound is subjected to a fast intestinal metabolism, causing the transformation of the chemical structure, as well as changes in its bioactivity (Dordević et al., 2015). This happens due to the isomerization of *trans*-resveratrol to *cis*-resveratrol.

Some researchers studied the stability and bioavailability of this bioactive compound and noticed that, after oral consumption of resveratrol, 70 % of the ingested amount had been absorbed into the body. However, only trace amounts of the free compound were found in the blood stream, which proves the

extensive intestinal metabolism (Koga, 2015; Koga, Andrade, et al., 2016). *Koga et al.* (2016) studied the effect of the encapsulated resveratrol on simulated gastro-intestinal digestion. More information is presented in section 2.6.

Considering all the reasons mentioned above, it is important to ensure the bioactivity of resveratrol when consumed and also a controlled release at the appropriated target (Dordević et al., 2015; Koga, Andrade, et al., 2016).

Furthermore, it is also important to try to mask resveratrol's taste, since this compound is associated with a feeling of bitterness on the food products to which it is added. This causes a bad acceptability of the products by the consumer (Koga et al., 2015). An example of this is the case of wine. Some tests were performed, verifying that the bitterness of this beverage increases when fortified with resveratrol (Koga et al., 2015).

One way to overcome these limitations presented by the compound would be to microencapsulate it. This way, it would be possible to protect it and preserve its stability and bioavailability. Additionally, it would allow to mask the bitter taste, prevent reactions with other components in the surrounding environment and control its release until the compound reaches the desired sites (Davidov-Pardo & McClements, 2014; Nedovic et al., 2011).

2.3 Health Benefits

Phenolic compounds are usually found in fruit and vegetables. They have attracted immense attention because many studies have shown that a diet rich in these food products plays an important role in the prevention of many diseases (Caruso, Tanski, Villegas-Estrada, & Rossi, 2004).

The food industry has tried to replace synthetic additives in food by bioactive natural products. However, they present some problems such as instability, react with other food matrix ingredients and exhibit strong odor and flavors (Dias et al., 2015).

Resveratrol is a polyphenolic compound, synthesized in a large number of plant species that is found naturally in the skin of red grapes, red wine, berries, peanuts and other peanut products in low amount (Koga, 2015; Zhang et al., 2013). The concentration of resveratrol found in literature, in red grapes, red wine and peanuts was 0.050 mg by 100 g, 0.002-0.653 mg L^{-1} and 0.002-0.0179 mg by 100 g, respectively (Koga, 2015). Chocolate was recently confirmed to also be a source of resveratrol (Shi et al., 2008).

Nowadays, consumers' concern about health issues is increasing due to the appearance of diseases which are related to poor eating habits and lifestyle of society (Dias et al., 2015).

The interest on this bioactive compound has grown, due to its potent antioxidant, anti-vital and anti-inflammatory properties and because it has been shown to have numerous health benefits with cardiovascular, cancer, diabetes and neurodegenerative diseases (Figure 2) (Trotta et al., 2015).

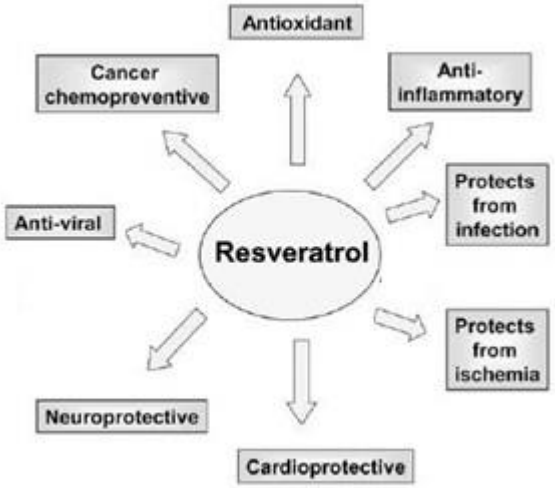


Figure 2 – Health benefits of resveratrol; Extracted from (Aggarwal & Shishodia, 2005).

Resveratrol's antioxidant properties play a significant role in the prevention of diseases associated with oxidative stress, such as cancer and neurodegenerative diseases. Degenerative diseases can appear when the cells are damaged by reactive oxygen species (ROS). Resveratrol has hydroxyl groups that possess redox properties and has a potential for electron delocalization across the chemical structure, which makes the compound act as an antioxidant, once it has the ability to scavenge ROS (Aggarwal & Shishodia, 2005).

In the oncological field, testes have been made about the effect of resveratrol against ovarian carcinoma cell lines. It was found that it inhibits the proliferation and survival of these cells (Koga, Andrade, et al., 2016). Other studies showed that this is also true for several types of human cancer cell lines, such as colon, skin, breast, lung, prostate, liver and pancreas. Resveratrol is also capable of inducing apoptosis and block cell cycle progression (Walle, Hsieh, Delegge, Oatis, & Walle, 2004).

Inflammation is considered a primary physiologic defense mechanism that helps the body to fight an infection and plays a major role in the pathogenesis of a wide variety of diseases (Aggarwal & Shishodia, 2005). Resveratrol has been shown to have anti-inflammatory activity, once it is able to suppress cyclooxygenase (COX)-2 expression. Activation of (COX)-2 leads to the production of prostaglandin E2(PGE2), which causes inflammation (Aggarwal & Shishodia, 2005; A. L. C. Alves, 2015).

Other studies made have also shown an anti-viral activity of the bioactive compound. Resveratrol is active, in particular, against viruses of the *herpesviridae* family. At least five species of this family are extremely widespread among humans, including HSV-1 and HSV-2 (*herpes simplex* virus type 1 and 2).

A published study demonstrated that resveratrol had the ability to inhibit the replication of the HSV-1 virus upon entry into the cell (A. L. C. Alves, 2015).

In the context of cardiovascular protection, the resveratrol molecule reveals very promising properties that contribute to a decreased risk of developing cardiovascular diseases. According to published *in-vitro* studies, resveratrol prevents platelet aggregation. The mechanism involved in this protective effect is based on the inhibition of enzyme COX-1 by resveratrol, which will promote blood flow and decrease clot formation (A. L. C. Alves, 2015).

Other studies about resveratrol have shown that it decreases blood glucose and insulin resistance, showing positive effects in diabetic males (Koga et al., 2015).

Therefore, with all the properties associated with this compound, it would be of great interest to add resveratrol into food products, helping to provide all its health benefits to the consumers and overcoming all its limitations (Zhang et al., 2013; Zupančič et al., 2015). However, its poor solubility in water also makes it difficult to incorporate high levels of resveratrol into aqueous-based food products (Davidov-Pardo & McClements, 2014).

2.4 Microencapsulation fundamentals

2.4.1 Some concepts

Microencapsulation is a technique in which a compound, liquid, solid, or gas, called active or core material, it is coated with a second material that will allow the formation of the wall (encapsulating agent), producing a capsule and protecting the core from the surrounding environment factors, such as light, heat, pH and moisture. The coating material is called carrier, shell, wall material or encapsulating agent (Carvalho, Estevinho, & Santos, 2016). An example of natural microencapsulation are biological cells, that protect the cellular part from the outside conditions and the cell membrane controls the release of metabolites (Yin, 2009).

Microcapsules can be classified according to their size and morphology. According to their size, they are called microcapsules if their diameters are in the range from 0.2 to 5000 micrometers. Capsules with diameters below 0.2 micrometers are called of nanocapsules and diameters greater than 5000 micrometers are called macrocapsules (Azeredo, 2005). This characteristic plays an interesting role in sensorial properties on food products, once it influences its texture (J. Aguiar et al., 2016).

According to their morphology, microcapsules are classified as shell type or as matrix type. In the shell type, there is one or multiple cores totally involved in a coating material (mononuclear and polynuclear). In the matrix type, the compound is homogeneously distributed within the shell material (Anandharamakrishnan, 2015; Jyothi, Seethadevi, Prabha, Muthuprasanna, & Pavitra, 2012). Microcapsules can present regular or irregular shape and also mononuclear with multi-shell

(Anandharamakrishnan, 2015; Carvalho et al., 2016). The structure, shape and size of the microcapsules depend on the encapsulating agent and the process of production. The morphologies described above are shown in Figure 3.

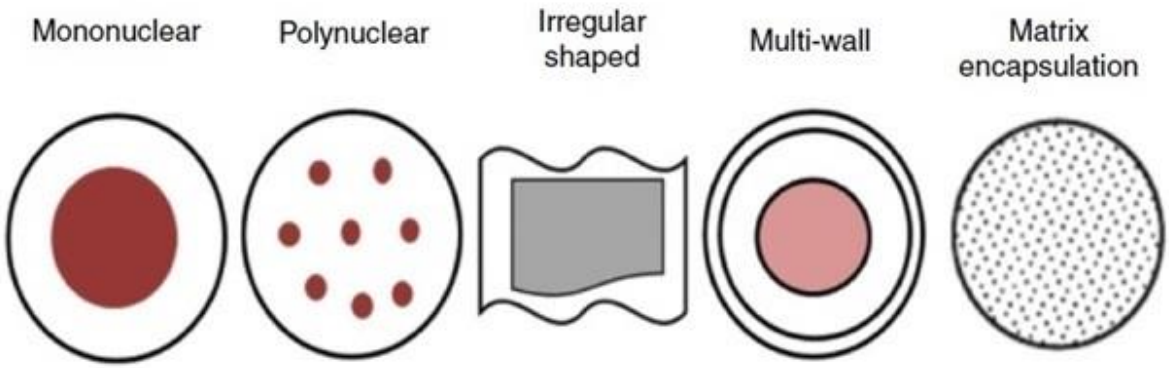


Figure 3 – Classification of microcapsules; Extracted from (Anandharamakrishnan, 2015).

Microencapsulation has a significant role in the food industry. It protects the core compound and controls its release to the outside. Moreover, this technique decreases the reactivity and incompatibility of the compounds with external factors, separate reactive or incompatible compounds, masks the taste of the encapsulated material and reduces the loss of nutritional value or aroma (Azeredo, 2005; Gonçalves, Estevinho, & Rocha, 2016; Jyothi et al., 2012). A common situation during the storage of some products is the loss of flavors due to the volatile properties of the compounds. In this case, microencapsulation is also a good solution to delay this situation (Gonçalves et al., 2016). The release control of the compounds encapsulated is one of the main advantages of this technique (J. Aguiar et al., 2016). However, there are several release mechanisms, according to the purpose of the encapsulated compound (Dubey, Shami, & Bhasker Rao, 2009).

The factor that will define the rate control of the compound is the permeability of the encapsulating agent (Jyothi et al., 2012). Impermeable walls are used when it is necessary to isolate active substances, followed by a fast release under defined conditions. The release by the increase of the outside pressure is one of those cases (Ghosh, 2006; Jyothi et al., 2012). On the other hand, permeable walls allow the controlled release of the active compounds that is required, for example, in the case of perfumes, to extend its release (Jyothi et al., 2012). In this case, the core release is controlled by the thickness of the encapsulating agent and its pore size (Ghosh, 2006).

2.4.2 Release Control of the core

The key functionality provided by the encapsulation technique is the release of compounds at controlled rates under specific conditions, in the right place and at the right time (Desai & Jin Park, 2005). This increases the process effectiveness and decreases the necessity to incorporate high doses of additives in food products, improving the cost of food production (Azeredo, 2005; Desai & Jin Park, 2005).

However, there are some factors that must be taken into account that affect the release rate and its effectiveness. The main ones are the interaction between the core and the encapsulating agent, core and encapsulating agent ratio, particles size and encapsulating agent viscosity (Azeredo, 2005).

There are many mechanisms that can activate the release such as, diffusion, solvent, biodegradation, pH, temperature and pressure (Azeredo, 2005; Dubey et al., 2009; Berta Nogueiro Estevinho et al., 2013). The choice of the right release mechanism and coating material provide an efficient release of the core (Dubey et al., 2009).

In the diffusion controlled release, the release is made by diffusion through the wall. The encapsulating agent acts as a semipermeable membrane and its rate it is controlled by the chemical properties of the core and microencapsulating agent, by the pores size and also by the wall thickness (Azeredo, 2005; Brasileiro, 2011; Yin, 2009).

The release by a solvent can be controlled by osmotic pressure (Brasileiro, 2011). In this case, the semi permeable polymeric membrane is permeable to the solvent, but not to the solute. The osmotic pressure difference, leads to a flow of fluid from the outside to the inside of the microcapsule, thereby forcing the saturated solution in the interior out of the capsule, through the pores (Gupta & Dey, 2013).

The mechanism of release by swelling is influenced by the physical state of the wall material. Glassy polymers are usually more impermeable than the ones in a swelling state (Azeredo, 2005).When a glassy polymer is compatible with a medium, the solvent penetrates the polymer matrix, suffering a transition to a swelling state with more mobility, thus releasing the core (Rao & Devi, 1988).

Biodegradation release occurs when the utilization of encapsulating agents can be degradable by the action of enzymes (Azeredo, 2005).

In the case of pH controlled release, the pH may cause changes in the solubility of the encapsulating agent. In a release that is controlled by temperature, there are two distinct situations. The release can be made by the collapse of the encapsulating agent when a critical temperature is achieved, or by fusion of the wall material (Azeredo, 2005; Brasileiro, 2011). Finally, the release by pressure occurs when the pressure is so high that the microparticles break. Chewing a gum is one of those cases (Azeredo, 2005).

According to the used encapsulating agent and the chosen method for microparticles production, different release profiles can be obtained.

2.4.3 Microencapsulation Agents

The selection of the adequate encapsulating agent is an important step in the microencapsulation process, to ensure its efficiency and the microcapsule stability (Davidov-Pardo, Arozarena, & Marín-Arroyo, 2013).

The selection is defined by microcapsules physical-chemical properties (Yin, 2009). It must be compatible with the core, considering its porosity and solubility and with the final destination of the microcapsule (J. Aguiar et al., 2016; Berta Nogueiro Estevinho et al., 2013). It needs to provide stability, strength and flexibility and be able to hold the core during the storage and processing (J. Aguiar et al., 2016; Desai & Jin Park, 2005; Dias et al., 2015).

Moreover, in microencapsulation process the components used as wall materials should be recognized as safe, the microencapsulation should not change organoleptic properties of the food products and the particles must remain stable during transportation and storage, keeping their metabolically active form (Davidov-Pardo & McClements, 2014).

Sometimes the use of only one encapsulating agent may not be enough if it does not have all the required characteristics. In this case, usually, a mixture of encapsulating agents can be used (Berta Nogueiro Estevinho et al., 2013).

Biodegradable polymers have been widely used in the food sector (Zuidam & Nedovic, 2010). Some of the most frequently used are carbohydrates, such as starch, modified starch and maltodextrins; gums, such as arabic gum, or alginates; proteins, such as gelatin or whey protein; and chitosan (J. Aguiar et al., 2016). In the next section, it will be described one of the most popular biopolymers used in microencapsulation processes – arabic gum.

2.4.3.1 Arabic Gum

Arabic Gum, also known as acacia gum, is a natural polysaccharide derived from Acacia species, that is abundant in central Sudan, central Africa and in West Africa (Dauqan & Abdullah, 2013). Arabic Gum is a complex polysaccharide composed by galactose, arabinose, ramnose, glicurônic acid and 2 % of a protein compound, covalently attached to the molecular arrangement. The last one plays a very important role in determining the emulsifying properties of the gum (Azeredo, 2005).

Arabic gum has emulsifier, binding agent and stabilizer properties in food and cosmetic products containing oil-water interfaces (Dauqan & Abdullah, 2013). It presents high water solubility, soft flavor, and relatively low viscosity, even in very concentrated solutions (Azzaoui, Hammouti, Lamhamdi, Mejdoubi, & Berrabah, 2014).

For all these reasons, arabic gum is an excellent encapsulating material. On the other hand, it has high cost and low availability once Acacia species are produced in Sudan regions where plantations are subject to unpredictable climatic variation (Azeredo, 2005; Martins, 2012).

Alginate or gelatin can also be a good solution to microencapsulation of food products (Berta Nogueiro Estevinho et al., 2013).

2.4.4 Microencapsulation Techniques

There are several different techniques for the encapsulation of compounds and many of them have been successfully used in the encapsulation of polyphenols (García, Vergara, & Robert, 2015). The most common techniques applied in the food industry are spray drying or spray cooling, fluidized-bed coating, spinning disk, coacervation and liposome entrapment (Berta Nogueiro Estevinho et al., 2013).

They can be divided into two different groups: chemical methods and physical-mechanical methods. The choice of the right technique is based on some parameters, such as the type of compound that is encapsulated, the encapsulating agent, the size of the microcapsule, production cost and the final use of the particles (Teixeira, 2010).

Some of the different encapsulation methods are presented in Table 1. Each one is briefly described according to the literature.

Table 1 – Most commonly used microencapsulation techniques in food industry; Extracted from (Berta Nogueiro Estevinho et al., 2013)

Microencapsulation Techniques	
Physical-Mechanical Methods	**Chemical Methods**
Spray-Drying	Coacervation
Spray-Cooling	Liposome entrapment
Fluidized Bed	
Spinning disk	

2.4.4.1 Spray-Drying

Spray-dryer is a physical-mechanical method successfully used in food industry that transforms liquids into powders. These are stable and easy to apply (Dordević et al., 2015). It is a low-cost technology, having lower production costs when compared with other methods, easy handling, continuous, rapid and easy to scale-up (Desai & Jin Park, 2005; Berta Nogueiro Estevinho et al., 2013; Nedovic et al., 2011). On the other hand, it also presents some limitations like low yields at laboratory scale and the particles are usually not uniform in terms of size and shape and have the tendency to aggregate (Dordević et al., 2015; Berta Nogueiro Estevinho et al., 2013).

This technique consists in the preparation of the feed solution, where the core particles are dissolved, emulsified, or dispersed in a polymer solution (Ghosh, 2006). It is very important to guarantee the homogenization of the solution before introducing it into the spray-dryer, so it does not interfere with the drying rate of the powder and ensure the efficiency of the process (Gonçalves et al., 2016). This solution is fed into the spray dryer and atomized into the heated chamber, allowing the evaporation of the water fraction present in the feed solution. This process makes the encapsulating agent solidify around the core particles, obtaining a powder that is collected in the bottom of the drier at a lower temperature, after separated by a cyclone (Dubey et al., 2009). Water evaporation occurs very quickly and the exposure time of the particles to the heat is short. This way, the probability of undesirable changes in thermosensitive compounds is low. With this technique, the capsules produced are usually matrix type (Azeredo, 2005). In Figure 4 is represented the spray-drying pathway.

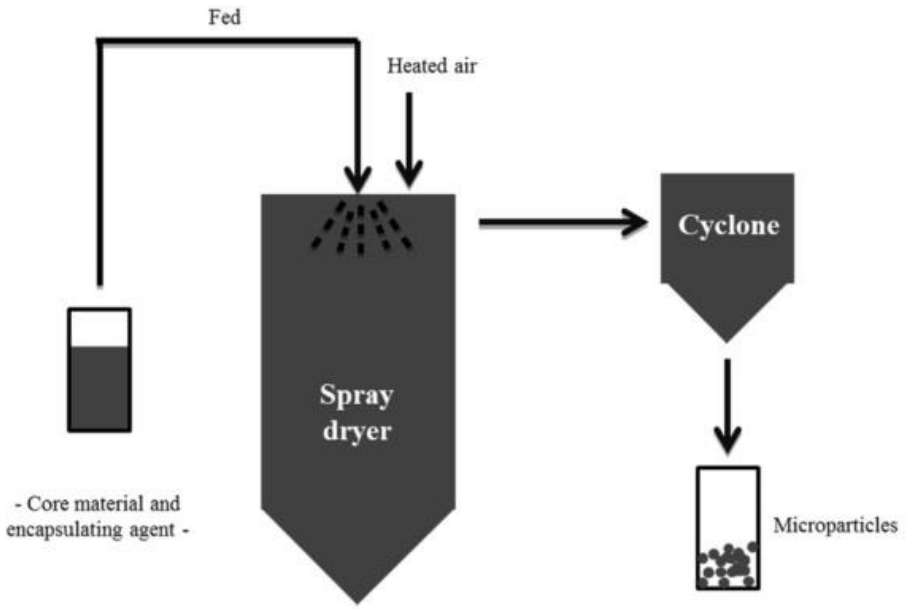


Figure 4 – Schematic representation of the spray-drying pathway; Extracted from (Gonçalves et al., 2016)

The spray-dryer should operate at optimal in and outlet temperatures. A variation in these conditions can cause problems in the process. The decrease of inlet temperature decreases the evaporation rate, resulting in particles with high water content and tendency to agglomerate. On the other hand, the increase of the inlet temperature leads to a faster evaporation, resulting in particles with fissures in the membrane (Gonçalves et al., 2016).

Some polyphenols and encapsulating agents used in spray-drying technique, found in literature, are mentioned in Table 2.

Table 2 – Encapsulation agent of different polyphenols using spray-drying technique

Polyphenol	Encapsulant	Technique	Ref
Anthocyanins	Maltodextrins	Spray-drying	(Bakowska-Barczak & Kolodziejczyk, 2011)
Catecin	Maltodextrins and Carboxymethylcellulose (CMC)	Spray-drying	(Boonchu & Utama-Ang, 2015)
Tannin	Maltodextrins and Carboxymethylcellulose (CMC)	Spray-drying	
Catechins	Mixture of maltodextrin (MD), mesquite gum (MG) and zein (Z)	Spray-drying	(Davidov-Pardo et al., 2013)
Proanthocyanidins	Mixture of maltodextrin (MD), mesquite gum (MG) and zein (Z)	Spray-drying	
Resveratrol	Sodium caseinate and whey protein concentrate	Spray-drying	(Koga, 2015)
Rosmarinic acid (RA)	Chitosan and modified Chitosan	Spray-drying	(Casanova, Estevinho, & Santos, 2016)
Epigallocatechin gallate (EGCG)	Hydrocolloid gums, inulin, pectin or alginate	Spray-drying	(Belščak-Cvitanović et al., 2015)
Gallic Acid (GA)	Cross-linked inulin (GA-CIn)	Spray-drying	(García et al., 2015)
Curcumin	Porous starch and gelatin	Spray-drying	(Wang, Lu, Lv, & Bie, 2009)

2.4.4.2 Spray-Cooling

Spray-Cooling technology is very similar with spray-drying. However, it does not involve evaporation of water to produce microcapsules and the encapsulating agent is usually vegetable oil or its derivatives, with melting points of 45 to 122 ºC (Desai & Jin Park, 2005). Due to the lipid wall, the microcapsules produced by this technique are insoluble in water and the release occurs normally directly in the intestine by the presence of lipases in the intestinal lumen (Dordević et al., 2015).

A hot melt homogeneous fluid, composed of the core and encapsulating agent are atomized into a cooled chamber and the wall solidifies around the core, producing dense and not porous microparticles (Lakkis, 2007). Spray-Cooling is often used to encapsulate food additives, such as vitamins, enzymes and flavors (Anandharamakrishnan, 2015; Garti & McClements, 2012).

This technique presents many advantages. It is a safe, rapid and easy to scale up the production once it can be operated in continuous (Dordević et al., 2015).

2.4.4.3 Fluidized Bed

Fluidized Bed coating consists essentially in the suspension of the core material in an air stream, at a predefined temperature. The core material is sprayed with a coating material and microparticles are formed by solidification of the encapsulating agent due to evaporation of the solvent (Dordević et al., 2015; Dubey et al., 2009).

2.4.4.4 Spinning Disk

Spinning Disk is a physical-mechanical method in which a suspension of core emulsified in encapsulating agent is prepared. The suspension is poured into a rotating disk and by the spinning action of the disk, the wall around the core material is formed. Coated particles are directed away from the disk axis by centrifugal force, where, usually by cooling, the wall material is solidified (Ghosh, 2006).

2.4.4.5 Coacervation

Coacervation is a chemical method that can be divided into two methods, complex and simple coacervation. In simple coacervation the separation of phases is achieved by addition of a desolvation agent. In complex coacervation, the separation is caused by the complexation of two polymers with opposite charges (Ghosh, 2006).

This technique involves three steps: formation of three immiscible phases – a liquid phase that acts as vehicle phase, a core material phase and a coating material phase; core's encapsulation; and stabilization of the microcapsules (Jyothi et al., 2012).

First of all, the core material is dispersed into a polymer solution. The second polymer solution is then added forming the three immiscible phases (Ghosh, 2006). The core's encapsulation process happens when the two polymers form a complex and this is triggered by some methods such as, changing temperature, pH, or addition of a salt (Jyothi et al., 2012). In the end, stabilization of the microcapsules is achieved by crosslinking, desolvation, or thermal treatment (Ghosh, 2006).

The process is schematically represented in Figure 5.

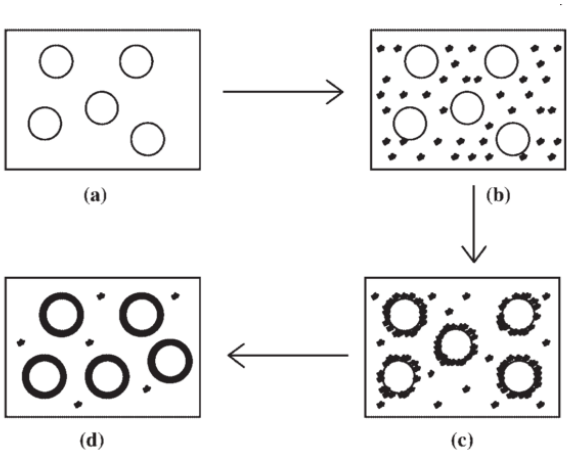


Figure 5 – Schematic representation of the coacervation process. (a) Core material dispersion in solution of wall material; (b) Separation of coacervate from solution; (c)coating of core material by coacervate;(d) coalescence of coacervate around core particles; Extracted from (Ghosh, 2006)

2.4.4.6 Liposome Entrapment

Liposomes are closed, continuous lipid bilayer structures formed by phospholipid molecules (Anandharamakrishnan, 2015).

These structures are able to encapsulate hydrophobic, hydrophilic and amphiphilic materials (Azeredo, 2005).

Liposomes are formed by hydrophilic-hydrophobic interactions between phospholipids and water molecules. The active core is entrapped within the aqueous compartment, or attached to the hydrophobic membrane bilayer of the liposome. The release of the core from the liposomes is triggered by a change in temperature and pH conditions (Zuidam & Nedovic, 2010).

Lately, the use of liposomes in the food industry has been suggested, especially for encapsulation or immobilization of enzymes (Azeredo, 2005).

2.5 Microencapsulation applications

Microencapsulation has many applications in several fields. It has been used in agriculture, pharmaceuticals, food industry, cosmetic and fragrances, textiles, coatings and many other industries.

2.5.1 Agriculture

Microencapsulation plays a significant role on chemicals to apply on agriculture, like pesticides and herbicides and had a great evolution due to the high concentration and toxicity of the products used in this field (Brasileiro, 2011).

Microcapsules are combinations of the pesticide with the encapsulating agent that protects it and releases it according to the necessity of controlling the pest. This technique has many advantages once it is safer to the environment, minimizes the exposure time of the workers and consumers and avoids flow of substances to water sources (Brasileiro, 2011).

2.5.2 Pharmaceutics

In pharmaceutical field microencapsulation is widely used for the controlled drug delivery (Dubey et al., 2009). It is used to prepare coated substances, so that the medicament will be selectively absorbed in a specific place. Beyond this, it also allows the separation of incompatible substances (Gupta & Dey, 2013).

2.5.3 Food Industry

Microencapsulation presents many applications in food industry as has been discussed throughout the project. It can overcome some problems like loss of some ingredients activity and its reaction with components present in the food system, that can limit the bioavailability (Dubey et al., 2009). This technology allows the companies to incorporate many beneficial substances in food products, like flavors, oils, enzymes, minerals, vitamins and fats to increase its durability and the substances storage time. Besides this, simplifies the food manufacturing process by converting liquids into solid powder (Brasileiro, 2011; Dubey et al., 2009).

For example, in the production of cheese, enzymes are microencapsulated to improve its flavor and, in the case of some candies, carbon dioxide can also be encapsulated to produce a sizzling effect on the tongue when the candy melts (Yin, 2009). Another application is related to the dried milk, where the milk fat is microencapsulated by a mix of lactose and milk proteins, protecting the core from oxidation (Berta Nogueiro Estevinho et al., 2013).

2.5.4 Cosmetic and fragrance

Essential oils are volatile oils extracted from plants that act as fragrance ingredients used in perfumes, cosmetics, soaps and other products. However, essential oils present some problems like having a short shelf life, since they are volatile. Microencapsulation plays a major role protecting and preventing the loss of volatile aroma compounds, controlling its release and its stability (Carvalho et al., 2016).

2.5.5 Textiles

In this field, the microcapsules are used to increase the duration of fragrances and skin softeners in textiles. Another application is the impregnation of insect repellents, vitamins, skin moisturizing agents, and anti-aging agents (Cheng, Yuen, Kan, & Cheuk, 2008; Nelson, 2002).

The compounds are impregnated in textiles and on contact with human body and skin, microcapsules rupture occurs through natural body movements and the cosmetic textiles release the active substance to the skin (Cheng et al., 2008).

2.5.6 Coatings

The coating is a material applied to a surface of an object with a decorative or functional purpose (Ghosh, 2006).

In coatings, microencapsulation can act as a protection technique. Microparticles incorporate the compound and they have enough strength to remain intact and collapse only when the coating is damaged releasing the content slowly over time. This way, it is possible to increase the material resistance (Dubey et al., 2009; Ghosh, 2006).

2.6 Resveratrol microencapsulation studies in the literature

There are many reports on resveratrol, most of them focused on its biological activities. Recently, studies have focused on the encapsulation of this compound to increase its stability and water solubility (Zhang et al., 2013). Most of the microencapsulation studies used emulsion-based delivery systems to encapsulate lipophilic compounds. However, there are already some studies using the spray-drying method, once it improves resveratrol's long-term stability by converting it into a powdered form (Davidov-Pardo & McClements, 2014).

Some of the reports on resveratrol's microencapsulation are described next.

Koga *et al*. (2016) studied the stability of encapsulated resveratrol to UV light and to simulated gastro-intestinal digestion. Whey protein concentrate and sodium caseinate were used as wall materials and the microencapsulation was made by spray-drying. Resveratrol's UV stability was evaluated using ratios of *trans:cis* resveratrol. Sodium caseinate microcapsules maintained higher ratio than whey protein concentrate microparticles, suggesting that sodium caseinate is a better encapsulation material. Digestive stability was higher using encapsulated resveratrol. Sodium caseinate microcapsules were again a better polymer providing a higher digestive stability and bioaccessibility. The authors concluded that resveratrol's encapsulation decreases the isomerization reactions of trans-resveratrol throughout digestion.

Zhang *et al.* (2013) studied the preparation of resveratrol nanodispersions to increase its stability to light and water solubility. The suspension was prepared by antisolvent precipitation process and then dried by spray-drying to generate resveratrol solid dispersion. In the end was verified that the resveratrol activity decreased only 14 w/w when exposed to light, while raw resveratrol decreased 80 w/w. Beyond this, encapsulated resveratrol dissolved completely after 45 minutes, while raw resveratrol, after 120 minutes, was not completely dissolved.

Salgado *et al*.(2015) tested resveratrol antifungal activity against *Botrytis cinerea*. To do so, an emulsion was formed and then spray-dried. β-glucans and soy lecithin were used as wall materials. The authors verified that, when applied pure resveratrol, no effect was observed on fungal growth. However, when applied encapsulated resveratrol, the growth was reduced between 50 and 70 %, concluding that encapsulation enhanced resveratrol antifungal activity.

Lee *et al.* (2013) studied the physicochemical and sensory properties of yogurt supplemented with microencapsulated peanut sprout extract, containing resveratrol, during storage at 4 ºC for 16 days. Microparticles were obtained by preparing a water-in-oil-in-water emulsion and then converted into powder, by spray-dryer. The authors concluded that at lower concentrations, powdered peanut sprout extract microcapsules can be efficiently used in yogurt production without affecting its properties, such as pH, viscosity, color and flavor. The lowest release was also obtained at lower concentration of microparticles. The release would affect the quality of the yogurt and resveratrol's properties.

Shi *et al.* (2008) studied the resveratrol's encapsulation by yeast cells for the first time, to achieve sustained release, stabilize the compound and improve its water solubility. To form the microcapsules, the authors used resveratrol, yeast cells, ethanol and water. These four components were mixed and stirred, forming a homogeneous solution. The cells were then centrifuged, the supernatant was decanted and the cells were freeze dried. In the end of this study, it was confirmed that yeast-encapsulated resveratrol enhanced stability and bioavailability of the compound, due the increased solubility of resveratrol. Beyond this, the release profile in simulated gastric fluid showed that about 90 % of resveratrol was released within 90 min. This confirms the increase of bioavailability of resveratrol, maintaining its biological activities.

Sanna *et al.* (2015) studied the effect of the concentration of chitosan and poly(D,L-lactic-co-glycolic acid), PLGA, to produce resveratrol's microcapsules. Microcapsules were formulated by Water/Oil/Water double emulsion technique. The release was made under simulated gastrointestinal fluids and stability of stored microcapsules was also monitored. The microcapsules resulted were shown to have encapsulation efficiencies of 40 to 52 %. The authors attribute the improvement of resveratrol encapsulation efficiency to the presence of hydrogen bonding between resveratrol and chitosan. Microcapsules with a greater concentration of chitosan exhibited a lower release of resveratrol, with only 40 % dissolved in the first two hours. Finally, it was verified a good protection of encapsulated resveratrol until 6 months, showing a good stability of stored microcapsules.

Hung *et al.* (2006) studied the incorporation of resveratrol into mixtures of emulsions and liposomes, to study its cardiovascular protection. The influence on the release of the compound in plasma was explored and it was verified that the inclusion of resveratrol retarded the release, in the presence and absence of plasma *in vitro*. Beyond this, the authors also studied the *in vivo* inhibition of neointimal hyperplasia by resveratrol, after arterial injury in a rat and it was verified that the injury was inhibited. Encapsulation efficiency obtain was 63 %.

Many other studies were made about encapsulation of resveratrol. Publications organized by method and encapsulating agent, are described in Table 3.

Analyzing the literature, it is possible to verify the existence of some studies about encapsulation of resveratrol. Applying the spray-dryer method, there are already few studies, but there is none using gum arabic as encapsulating material, thus being a progress in this field, considering all the biological advantages of this encapsulating agent.

Table 3 – Studies about microencapsulation of resveratrol.

Method	Encapsulanting Agent	Objective	Results	Ref
Emulsification	Chitosan	Enhance resveratrol *in vivo* skin penetration	Significant enhancement of resveratrol penetration	(Scalia, Trotta, Iannuccelli, & Bianchi, 2015)
Spray-Dryer	Dipalmitoylphosphatidylcholine (DPPC) and hyaluronic acid (HA)	Produce a system for use in chronic wound healing treatment	Presence of resveratrol decreases oxidation in cells	(Eroğlu et al., 2015)
Oil-in-water emulsion and solution – evaporation methods	Poly(lactic-co-glycotic acid) (PLGA)	Evaluate resveratrol release in interleukin-1β	Resveratrol microsphere is a promising candidate for cartilage repair and osteoarthritis therapy	(Wu, Wang, Li, Ke, & Yao, 2016)
Emulsion/Solvent Evaporation	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and poly(ε-caprolactone)(PCL)	Evaluate the feasibility of resveratrol's microcapsules as an oral drug delivery carrier	Feasible oral drug delivery carrier, being an attractive alternative in chronic diseases prevention	(Mendes et al., 2012)
Spray-Dryer	Sodium Caseinate	Evaluate consumers acceptance of resveratrol in easy-to-consume food products	Addition of encapsulated resveratrol did not effluenced the acceptance of bars, but decreased the acceptance of gummies	(Koga, Lee, & Lee, 2016)
Spray-Dryer	poly(ε-caprolactone)(PCL)	Development of microparticles to suitable drug deposition on the lungs	Microparticles possessed properties suitable for drug deposition on the bronchial and alveolar regions of the lungs. Results opened perspectives to further investigation.	(Dimer, Ortiz, Pohlmann, & Guterres, 2015)

3 Technical description

In this section, the procedures, either of production and characterization of microcapsules, are presented.

3.1 Microcapsules Production – Method

3.1.1 Reagents

Arabic gum was acquired from Fluka, Germany (30888-1KG, Lot # BCBK8649V), resveratrol was purchased from Sigma-Aldrich, USA (R5010-100MG, Lot # SLBS1063V) and coconut oil was obtained from Sigma-Aldrich, USA (C1758-500G, Lot # MKBW4734V). Millipore™ water purification equipment was acquired from Massachusetts, USA, and water was deionized in the laboratory.

3.1.2 Validation of analytical method

Validation of analytical method was made by UV/Vis Spectrophotometry (SPEC RES+, Sarspec, Porto, Portugal). Eight calibration standards solutions were prepared for resveratrol (from 0.2 to 6.12 μg mL^{-1}), at a wavelength of 308 nm. Each solution was analyzed in triplicate and a calibrations curve was obtained. For the preparation of the standards, a resveratrol solution was prepared. It was composed by 100 mL of coconut oil and 10.2 mg of resveratrol.

The method was validated in terms of accuracy, precision, linearity range, detection limit, quantification limit and specificity. The calibration curve is considered appropriated for analysis if it has at least 5 different standards solutions, with linearity range factor greater than 10 and correlation coefficient greater than 0.995.

3.1.3 Spray-Dryer Technique

The mini spray-dryer BÜCHI B-290 (Flawil, Switzerland), with a standard 0.5 mm nozzle, was used for the microencapsulation process. Resveratrol's solution was prepared in the same day as the spray-drying and the encapsulating agent's solution (arabic gum) was prepared in the day before. Arabic gum solutions were prepared in 100 mL of ultra-pure water and with concentrations of 10, 15 and 20% (w/V). Two types of microcapsules were prepared: (1) microcapsules with arabic gum, coconut oil and resveratrol and (2) microcapsules with only arabic gum.

Approximately 30 minutes before the spray-drying, 2 mL of the resveratrol solution was added to 100 mL of encapsulation agent solution, at room temperature and then fed to the equipment. The final solutions were stirred at 12 500 rpm, for 5 minutes, in the homogenizer to obtain an emulsion.

Thus, the core and encapsulating agent mass ratio was 1:50 (w/w). The emulsions were fed to the spray-dryer at room temperature. The same procedure was followed for all the samples.

The encapsulation process conditions used, for all samples, were air flow rate of 100 %, solution flow rate of 15 %, air pressure of 5-6 bar, inlet and an outlet temperature of around 150 ºC and around 77 ºC, respectively. The spray-dryer conditions were selected based on a previous study where the same encapsulating agent and the same oil was used (Gonçalves, Estevinho, & Rocha, 2017).

After being encapsulated, microcapsules were collected and stored under refrigeration in flasks that did not allow the passage of light, in order to avoid changes in product characteristics.

3.1.4 Product yield

The product yield (PY) of the spray-drying process was obtained, for all types of particles produced, by the ratio between the mass of powder obtained in the final of the process and the solid content of the feed solution. Product yield was calculated applying the Equation 1.

$$PY(\%) = \frac{mass\ of\ output\ powder}{solid\ content\ of\ feed\ solution} \times 100 \qquad (Eq.1)$$

3.2 Characterization of the microcapsules

3.2.1 Scanning electron microscopy (SEM)

Microparticles surface structure was performed by SEM analysis in a Fei Quanta 400 FEG ESEM/EDAX Pegasus X4M (Eindhoven, The Netherlands). Particles were fixed by an adhesive tape and then coated with a thin film of gold, at Centro de Materiais da Universidade do Porto (CEMUP), for further observation.

3.2.2 Particle size distribution

Microparticles size distribution was measured in Coulter LS 230 Particle Size Analyzer (Coulter, Miami, FL), by laser granulometry.

For each experiment, microcapsules were fed to the equipment with ethanol as solvent. The measurements were made in three runs of 30 seconds and characterized by number and volume.

Microparticles size distribution was measured for particles with arabic gum, coconut oil and resveratrol, and for particles with only arabic gum.

3.2.3 Controlled Release Studies

The release profile of resveratrol encapsulated with arabic gum was performed for the microparticles prepared with three different concentrations of encapsulating agent: 10, 15 and 20% (w/V). The execution of assays was made by adding 20 mg of microparticles on top of 3 mL of coconut oil, at 37 ºC, under continuous stirring and protected from light. The tests were performed in triplicate in separated samples during 30 minutes, in a continuous mode. The absorbance was read at intervals of 30 seconds, at 308 nm. After the defined time interval, it was possible to obtain the release profiles for each solution of encapsulating agent. The release profiles were quantified by UV-Vis spectrophotometry method.

3.2.3.1 Entrapment efficiency (EE)

The entrapment efficiency (EE) was obtained, for all types of particles produced, by the ratio between the amount of compound inside the particles and the total amount of core. The amount of core inside the particles was calculated subtracting the release value at time zero that corresponds to the amount of core that was not microencapsulated, from the value of the total release. Therefore, the entrapment efficiency was calculated applying the Equation 2.

$$EE(\%) = \frac{Total\ amount\ of\ core\ released - amount\ of\ core\ released\ right\ after\ dispersion}{Total\ amount\ of\ core\ released} \times 100 \quad (Eq.2)$$

4 Results and Discussion

Initially, the analytical method used in this work was validated. UV-Vis spectrophotometry was used to do the calibration curve and for quantification of resveratrol. Validation purpose was to ensure the ability of the method for the quantification of the bioactive compound and guarantee good measurements. The analytical method was validated regarding linearity range, accuracy, precision, detection limit, quantification limit and specificity. The results are presented in section 4.1. Additional information can be consulted in Appendix A - Validation of the analytical method.

Different types of particles containing resveratrol and empty particles were produced by the spray dryer method, using arabic gum as encapsulating agent. After this process, it was possible to calculate the product yield (PY) for each type of particles. Results are presented in section 4.2.

After microencapsulation, characterization of the particles was required. They were characterized by size and shape, once these features have an impact on their application in food products. Obtained results are presented in section 4.3.

Controlled release studies were also analyzed. The release was performed with microparticles prepared with three different concentrations of encapsulating agent: 10% (w/V), 15% (w/V) and 20% (w/V). After obtaining the release profiles it was also possible to calculate the encapsulation efficiency (EE). Results are presented in section 4.4.

Finally, Weibull and Korsmeyer-Peppas kinetic models were studied, applying mathematical models to the release profiles obtained experimentally. Results are presented in section 4.5.

4.1 Validation of analytical method

Information taken from the calibration curve is used to quantify the compound in unknown samples. However, it also permits to discover the quantification parameters that allow the validation of the method.

The analytical method was validated in terms of linearity range, detection limit, quantification limit, precision, accuracy and specificity. This way, resveratrol's calibration curve was linear between 0.2 μg mL^{-1} and 6.1 μg mL^{-1} and a linear regression was obtained (Eq. 3).

$$Abs = (0.065 \pm 0.0059)C(\mu g\, mL^{-1}) + (0.086 \pm 0.016) \qquad (Eq.3)$$

In order to obtain the calibration curve, eight standard solutions were prepared and a correlation coefficient of 0.9916 was obtained, which means that the experimental values fit a linear regression. The values of detection limit and quantification limit obtained were 0.31 μg mL^{-1} and 1.0 μg mL^{-1}, respectively. These two parameters were determined from the calibration curve.

Precision was expressed in terms of the repeatability. Repeatability is expressed by the standard deviation of several determinations of the same sample (A. Alves, 2016). Precision value obtained, for the standard of 6.1 μg mL^{-1} was 2.8 %, and for the standard of 2.0 μg mL^{-1} was 5.3 %.

Accuracy was obtained by the ratio between the concentration expected for each standard solution of resveratrol and the concentration obtained experimentally. Accuracy value obtained for the three determinations of theoretical concentration for the standard solution of 6.1 μg mL^{-1} was 97 %, and for the standard of 2.4 μg mL^{-1} was 109 %.

Specificity was evaluated by the ability of the method to detect resveratrol and the encapsulating agent. Resveratrol was measured around 307 nm and Arabic gum around 226 nm.

4.2 Product Yield

To evaluate the spray dryer method, it is very important to measure the product yield. This parameter allows the comparison between the amount of powder that is collected in the bottom of the drier and the raw material used. Table 4 presents the results obtained for the different microcapsules.

Table 4 – Product yield results

Microparticles formulations	Product Yield (%)
10 % AG only	43.5
15 % AG only	47.1
20 % AG only	51.7
10 % AG + Coconut oil + RSV	16.7
15 % AG + Coconut oil + RSV	25.8
20 % AG + Coconut oil + RSV	22.7

Analyzing the product yield results is seen that there is a big difference between the values obtained for the empty particles and the load particles. For microparticles composed only of arabic gum the product yield varied from 43.5 to 51.7 %, while for microparticles composed by arabic gum, coconut oil and resveratrol, the product yield varied from 16.7 to 25.8 %. Similar results were found by *Gonçalves et al.* (2017) for load particles, using the same oil and encapsulating agent. On the other hand, for non-loaded particles, *Gonçalves et al.* (2017) got higher values. In general, increasing the amount of gum used the product yield increases as well.

These values of product yield can be explained by the observation of powder deposited on the walls of the spray dryer, which causes many losses in the process. Load microparticles present significantly lower values than the non-load particles, which can be explained by the presence of coconut oil that increases the adhesion to the equipment walls.

Conditions of operation of the spray dryer and the size of microparticles can also influence the product yield values of the process. The inlet temperature is a very important parameter, once if it decreases, the evaporation rate decreases as well, resulting particles with high water content and tendency to agglomerate. On the other hand, the increase of the inlet temperature leads to a faster evaporation, resulting in higher product yields, once fewer particles will stay in the walls of the equipment (Gonçalves et al., 2016). For this reason, the choice of the adequate conditions in the equipment is very important to

obtain microparticles with the proper characteristics. Relatively to the size of the particles, when they are too small they can be aspired along with the air that leaves the spray dryer, instead of following to the collector (Joana Aguiar, Costa, Rocha, Estevinho, & Santos, 2017).

4.3 Microparticles Characterization

4.3.1 Particle Size Distribution

Different microcapsules were produced by spray drying. It was produced microcapsules of arabic gum containing resveratrol dissolved in coconut oil, so it would be possible to evaluate the effect of the concentration of encapsulating agent in incorporation and protection of resveratrol and microcapsules composed only by arabic gum.

Considering food applications, the particle size is a very important parameter, once it interferes with sensorial properties on food products, such as texture (J. Aguiar et al., 2016). Besides this, it also affects the release.

Particle size distribution was evaluated by volume and number, in Coulter LS 230 Particle Size Analyzer (Coulter, Miami, FL), by laser granulometry. The results are presented in Table 5.

Table 5 – Mean diameter of microcapsules by laser granulometry analysis, prepared with different formulations, considering differential volume and number distribution.

Samples	**Mean diameter (μm)**	
	Differential volume	Differential number
10 % AG only	7.3 ± 4.2	0.10 ± 0.11
15 % AG only	6.8 ± 4.1	0.21 ± 0.24
20 % AG only	7.8 ± 5.1	0.12 ± 0.15
10 % AG + Coconut oil + RSV	8.2 ± 4.8	0.26 ± 0.28
15 % AG + Coconut oil + RSV	8.1 ± 4.9	0.25 ± 0.30
20 % AG + Coconut oil + RSV	8.2 ± 4.8	0.72 ± 0.65

Analyzing the results obtained for all the particles is verified that, considering volume distribution, the average diameter of the obtained non-load microparticles ranged from 6.8 μm to 7.8 μm, while load particles got an average diameter of 8.2 μm for all the concentrations of encapsulating agent used. Considering number distribution, the average diameter obtained for non-load particles varied between 0.10 μm and 0.21 μm, while for load particles it ranged from 0.25 μm to 0.72 μm.

In terms of size of the particles, there is not a clear tendency of this parameter. For non-load particles, regarding differential volume, the higher value was obtained using 20% (w/V) of arabic gum, while the lowest value was correspondent to 15% (w/V) of encapsulating agent. Regarding number distribution, the higher and lowest value was obtained using 15% (w/V) and 10% (w/V) of arabic gum, respectively. On the other hand, for load particles, regarding differential volume, the average size obtained for all concentration of wall material was constant, being verified a significant increase of the average diameter of the particles, regarding number distribution, when using 20% (w/V) of arabic gum. This suggests the heterogeneity of the particles size formed by the spray dryer process, which can also be observed in the SEM images, in section 4.3.2.

Beyond this, it was concluded that particles composed only by arabic gum got lower diameter values compared with particles containing coconut oil and resveratrol. This means that the bioactive compound, and the coconut oil influence, in some way, the size of the particles formed, since viscosity will increase in load particles and, consequently, bigger particles will be produced.

Finally, the values obtained in Table 5 may suggest some aggregation of the particles during the spray dryer process. Agglomeration of the particles is a problem in the production of microcapsules with applicability in food industry, since it is not desired when it is intended to introduce the particles in the final product. In food products, the homogeneous size of the particles is crucial to ensure consistency and similarity of the products (Joana Aguiar et al., 2017).

4.3.2 Scanning Electron Microscopy (SEM)

Microparticles with and without resveratrol, were analyzed. The samples were prepared and then their surface was evaluated by scanning electron microscopy. The images are presented in Figure 6.

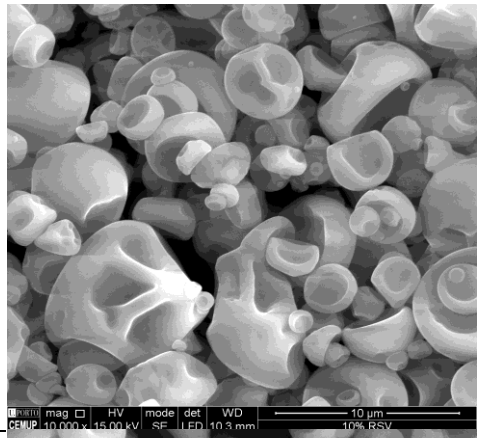
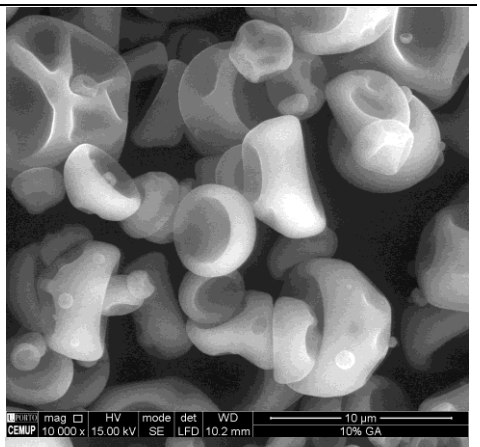
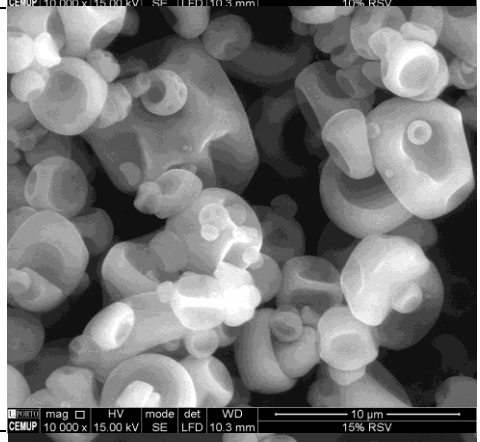
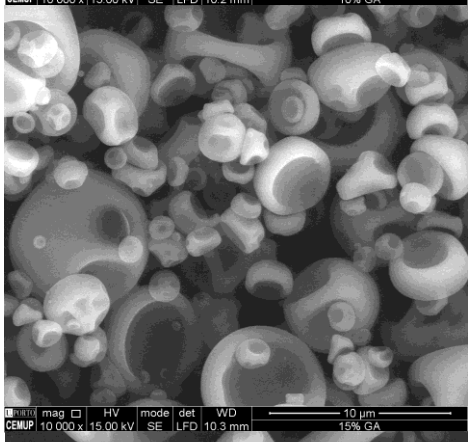
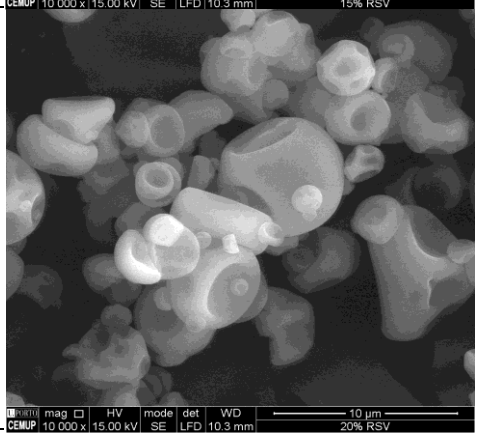
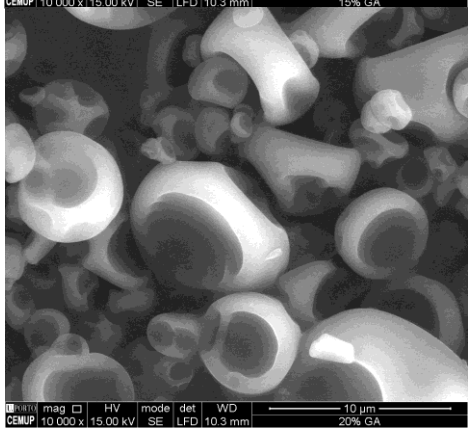
Arabic Gum	With Resveratrol	Without Resveratrol
10% (w/V) arabic gum		
15% (w/V) arabic gum		
20% (w/V) arabic gum		

Figure 6 – SEM images of arabic gum microcapsules with resveratrol and without compound. Amplified 10 000 times, beam intensity (HV) 15.00 kV, distance between the sample and the lens (WD) around 10 nm.

Analyzing SEM images, it is possible to confirm the formation of microcapsules, which allow us to claim that it is possible to encapsulate resveratrol by the spray dryer method. Therefore, all microparticles presented the same surface structure, with a spherical form and irregular shape, regardless of the concentration of wall material used.

Besides this, particles do not present cracks or breaks. This characteristic is fundamental to ensure a good protection and retention of the compound. The irregular shape observed in the images can be a result of a fast evaporation in the spray dryer, causing a rapid contraction of the particles.

Similar results were found by *Gonçalves et al.* (2017) in the microencapsulation of Vitamin A with the same oil and biopolymer. Microcapsules with the same characteristics were also obtained by *Estevinho et al.* (2014), that encapsulated β-galactosidase by spray drying using arabic gum as encapsulating agent.

4.4 Controlled Released Studies

The protection of resveratrol is very important due to its health benefits, being necessary to preserve its properties. For this reason, it is essential to develop delivery systems able to protect this bioactive compound after oral administration. Resveratrol microparticles, are capable of control its release and improve its bioavailability.

The controlled release studies of resveratrol were performed in coconut oil, in assays of thirty minutes, with microparticles prepared with three different concentrations of arabic gum (10% (w/V); 15% (w/V); 20% (w/V). The obtained release profiles for the resveratrol microcapsules are shown in Figure 7. The results profiles are presented in terms of release percentage over time, normalized by the maximum concentration of resveratrol released.

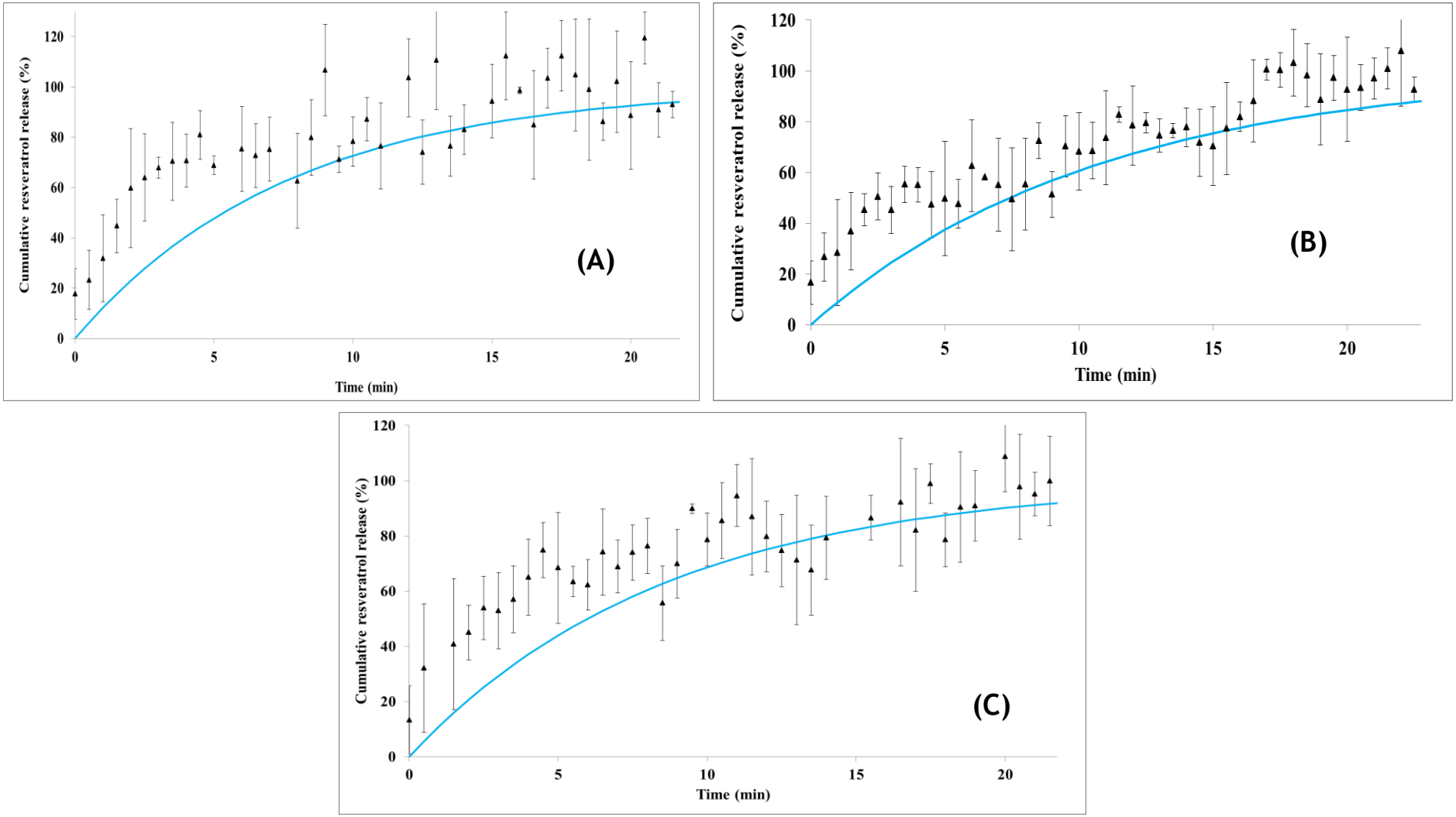


Figure 7 – Experimental release profiles of resveratrol microparticles in coconut oil: (A) 10% (w/V) GA; (B) 15% (w/V) GA; and (C) 20% (w/V) GA; and theoretical release profiles obtained by Weibull kinetic model.

Analyzing the release profiles for all concentrations of encapsulating agent is possible to see that all microcapsules tested present a similar behavior. In the first 15 minutes, there is an increase of the release, after which is verified that it begins to stabilize. This means that the complete release of resveratrol to the surrounding environment was achieved and that the presence of resveratrol in the microcapsules was confirmed. The fast release of the compound in the beginning can be related to the low amount of compound that was possible to encapsulate. *Gonçalves et al*.(2017) studied the release profiles of vitamin A in the same conditions as the present study. Similar release profiles were obtained, with a total release of the compound after 20 minutes, for concentrations of 10% (w/V) and 15% (w/V) of arabic gum. From microcapsules made of 20% (w/V) of wall material, the total release took 40 minutes to be achieved.

The concentration of arabic gum used influence the protection and stabilization of resveratrol and also its release. Good results were obtained for all concentrations used. However, for 10% (w/V) and 15% (w/V) of arabic gum, there was no significant difference in terms of protection of the core, once in both cases, at time zero, the percentage of compound detected was practically the same. For 20% (w/V) of arabic gum, the amount of compound detected was lower, which means that with this concentration of encapsulating agent, it is possible to have a better protection of the core. Microcapsules produced by spray drying are usually of matrix type, so the compound is homogeneously or heterogeneously distributed within the shell material and so there may be compound very close to the surface of the particle, contributing, as well, to the increase of the initial value. In terms of release, for all the concentrations of wall material used, it was concluded that it did not interfere with the release time, once in all cases, the total release was achieved at the same time, approximately.

The main results obtained in this study allow to conclude that the microcapsules produced by spray drying are effective in the protection of resveratrol and that arabic gum is a good encapsulating agent, being one of the most commonly used due its emulsifying and excellent retention properties during drying (De Barros Fernandes, Borges, & Botrel, 2014). Besides this, encapsulating agent influences the release profiles, being its choice a very important step on the production of microparticles. Depending on the desired controlled release, a different wall material can be chosen. Previous studies show how the encapsulating agent allows to obtain different release profiles for the same compound. For example, *Estevinho et al.* (2016) studied the influence of different biopolymers in the release of vitamin B12 and vitamin C and observed a total release of the vitamin encapsulated by chitosan after 120 minutes. For microcapsules produced with alginate, the released took 15 minutes and 10 minutes when using modified chitosan.

The release was made, experimentally, in coconut oil. Arabic gum has groups with strong affinity for oil, being able to swell in contact with it, forming a gel and by this way release the core compound.

4.4.1 Entrapment efficiency (EE)

The controlled release studies enable, as well, the determination of the entrapment efficiency of the bioactive compound. This parameter measures the percentage of compound that was encapsulated and it was calculated by the ratio between the total amount of resveratrol inside the microcapsules and the amount of compound detected in the solution, at time zero (Eq.2). Entrapment efficiency values obtained for all the three concentrations of encapsulating agent used are presented in Table 6.

Table 6 – Entrapment Efficiency results

Microcapsules formulation	Entrapment Efficiency (%)
10 % AG + Coconut oil + RSV	82
15 % AG + Coconut oil + RSV	83
20 % AG + Coconut oil + RSV	87

For 10% (w/V) and 15% (w/V) of arabic gum, the values of entrapment efficiency obtained were practically the same, increasing with the highest concentration tested. These results show that an increase of the concentration of wall material implies an increase of the encapsulating efficiency and thus improves the stability of the encapsulated compound, which confirms the conclusions reached earlier. Greater amount of arabic gum means that there is more encapsulating agent available to encapsulate the core material.

4.5 Kinetic Models

Different release profiles can be obtained according to the encapsulating agent used, the method applied for the production of the capsules and the conditions selected for the release. There are numerous models that can describe the release of bioactive compounds (Gonçalves et al., 2016).

The models applied to the release profiles obtained experimentally in this work were the Weibull model (Eq.4) and the Korsmeyer-Peppas model (Eq.5). The Weibull model was applied for being the most suitable for comparing release profiles of matrix-type capsules (Berta N. Estevinho & Rocha, 2016). This model is based on different mathematical functions that describe the release. This way, the release of encapsulated resveratrol over time, using three different concentrations of encapsulating agent was studied applying the Equation 4.

$$M_t = M_\infty \left[1 - e^{-\left(\frac{t-t_0}{\tau_d}\right)^\beta}\right] \qquad (Eq.4)$$

Where M_t is the percentage of compound release at time t (min), M_∞ is the percentage of total release, t_0 is the lag-time (min) of release, and is usually zero, β is the parameter that represents the shape of the release curve and τ_d is the time (min) when 63.2 % of Mt has been released.

The experimental data was adjusted in a linearized equation of the Weibull model and the main parameters of the model, the constants β and τ_d, were estimated. Subsequently, it was applied the Equation 4 and the theoretical release profiles were obtained. These are represented in Figure 7, by blue color. The values of the main parameters are presented in Table 7.

Table 7 – Kinetic parameters of the Weibull model applied to the resveratrol release from arabic gum microcapsules.

Arabic Gum	$\tau_{d\ (min)}$	$\tau_{d\ model\ (min)}$	**β**	R^2
10% (w/V)	8.0	3.8	0.491	0.835
15% (w/V)	6.0	6.5	0.604	0.817
20% (w/V)	6.0	4.3	0.500	0.820

According to Table 7, regression coefficient (R^2) values obtained for all the samples were very good taking into account that the system of release was very unstable.

About the parameter β, when it takes values higher than 1, it means that the shape of the curve gets sigmoidal with a turning point. On the other hand, if β is equal or lower than 1, the shape of the release curve matches with an exponential profile, but with a steeper increase in the case of β lower than 1 (Berta N. Estevinho & Rocha, 2016). The experimental values obtained to this parameter are all lower than the unity, meaning that the release rate decreases with time.

The constant τ_d, relative to time when 63.2 % of the compound has been released, was obtained experimentally and was calculated by the model. Comparing the values, it is possible to conclude that they were relatively close, for all the three different concentrations of encapsulating agent used in the preparation of the microparticles. This, corroborates, the good fit of the model to the experimental data.

Therefore, analyzing Figure 7 and all the parameters of the model obtained, we can conclude that the Weibull model fits perfectly in the results obtained experimentally, being suitable to describe the release behavior of resveratrol.

The main mechanism involved in the release of resveratrol was by swelling of the biopolymer used to produce the microcapsules.

The model of Korsmeyer-Peppas was also applied using the Equation 5.

$$\frac{Q_t}{Q_\infty} = K_k t^n \qquad (Eq.5)$$

Where Q_t/Q_∞ is the fraction of active compound release until time *t*, K_k is the Korsmeyer constant, and *n* is the release exponent, that defines the release mechanism.

The experimental data was adjusted to the equation 5, and the main parameters of the model were obtained. The values of the main parameters are presented in Table 8.

Table 8 – Parameters and correlation coefficients of the Korsmeyer-Peppas model applied to the experimental data

Arabic Gum	$\mathbf{K_{k\,(min^{-n})}}$	**n**	$\mathbf{R^2}$
10% (w/V)	0.378	0.0793	0.580
15% (w/V)	0.408	0.120	0.779
20% (w/V)	0.438	0.151	0.936

According to Table 8 the Korsmeyer-Peppas model does not fit so well to the experimental results obtained. The exception is for the microparticles prepared with 20% (w/V) of arabic gum, where is verified a good regression coefficient (R^2). The parameter *n* defines the mechanism responsible for the release. The release can be associated with Fickian diffusion mechanism (*n* lower than 0.43). The constant K_k increased with the increase of the amount of Arabic gum used to prepare the microparticles.

Many other authors have been applying the kinetic models. *García et al.* (2015) studied the production and characterization of microcapsules of gallic acid by native, cross-linked and acetylated inulin and concluded that both Higuchi and Peppas models were the ones that best fitted the experimental release profile. These models predicted a release mechanism by diffusion.

Concluding, even that Weibull and Korsmeyer-Peppas model are discussed in this project, there are many kinetic models to describe the different types of release. They present an acceptable approach to the

real systems behavior, helping to predict in vivo bio-performances and thus, avoiding the waste of time and money developing products that will not have the desired behavior when applied to the final goal.

5 Conclusions

This project's purpose was to produce and characterize microcapsules prepared with different concentrations of arabic gum (10, 15 and 20 % (w/V)), containing resveratrol by spray drying technology. In addition, it was also proposed the application of mathematical models to the experimental release profiles.

The product yield was calculated after the spray-drying process and the values obtained were satisfactory, considering the scale used. Microparticles composed only by arabic gum got higher product yield compared with load microparticles. The higher value obtained for non-load particles was 51.7 %, while load particles got 25.8 %. The product yield increased when using higher amounts of coating material for empty particles. However, to microcapsules containing resveratrol and coconut oil, that did not happen, suggesting that the oil increases the adhesion of the capsules to the walls of the equipment. Regarding particle size distribution, microparticles load with resveratrol presented mean diameters around 8 μm, considering volume distribution, while non-load microparticles presented mean diameters around 7 μm. This means that the coconut oil affects the size and product yield of the process. All microparticles surface was evaluated by scanning electron microscopy, showing spherical form and irregular shape, regardless of the concentration of wall material used. Controlled release profiles presented a similar behavior and the total release time was 15 minutes for essays using microparticles prepared with 10 and 15% (w/V) of arabic gum and around 20 minutes for the essays using solutions with 20% (w/V) of wall material. Results show the ability of the system of the compound's total release. The highest encapsulation efficiency obtained was 87 %, for the microparticle prepared with 20% (w/V) of encapsulating agent. Finally, the release profiles fitted well in the Weibull model.

Results suggest that microcapsules produced are effective in the protection and stabilization of the active form of resveratrol, being a good carrier for this compound and a promising delivery system for it in food industry.

6 Evaluation of the work

6.1 Accomplished goals

The goals accomplished were the encapsulation of resveratrol by spray-drying method and characterization of the microcapsules obtained. The application of mathematical models to the experimental release profiles, for all the concentrations of encapsulating agent used, was also an accomplished goal in this project.

6.2 Limitations and Future work

The main limitation of this project was the availability of the equipment, namely the spray dryer and the UV-Vis spectrophotometer. This limited the time spent in the laboratory and the quantity of results obtained.

For future work, it would be interest to storage the microparticles obtained for some months and then re-evaluate the release. This would allow old and fresh samples to be compared and also to evaluate their stage of degradation.

The study of microencapsulation using different encapsulating agents would be interesting as well. This way, it would be possible to evaluate the effect of different biopolymers, in terms of protection and stability, in the encapsulation of the bioactive compound.

A deeper characterization of the microparticles could also be made, by performing Fourier transform infrared spectroscopy (FTIR) analysis, in order to better understand the interaction between the core and the encapsulating agent.

One last way to improve this investigation could be by evaluating of the effect of pH in the controlled release.

6.3 Final appreciation

The time in Laboratory of Process Engineering, Environment, Biotechnology and Energy (LEPABE) was a path of learning and continuous improvement. During these five months, this dissertation has enabled me to develop important skills such as critical and logical thinking. It has also given me deeper knowledge in the field of microencapsulation and new knowledge in understanding and handling the equipment used. Last, but not least, all the laboratory colleagues were always very kind and helpful, making the time spent there much more pleasant.

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Appendix A - Validation of the analytical method

The validation of the analytical method consists in obtaining a set of statistical parameters that characterize it and that allow proving that the method is valid (A. Alves, 2016). Therefore, it is possible to quantify the resveratrol and obtain accurate and precise results for the intended use.

For the validation were calculated parameters of characterization, quantification and reliability of the method. Characterization parameters tell what the method is for (specificity). Quantification parameters encompass the study of linearity and associated statistical parameters (slope, interception, correlation coefficient, detection and quantification limits). Reliability of the method parameters show if the method is suitable for the obtained results (precision, accuracy) (A. Alves, 2016).

Detection limit (LOD) is the minimum concentration from which it is possible to detect the presence of the desired compound. On the other hand, the quantification limit (LOQ) is the lowest concentration of the desired compound that is possible to measure (A. Alves, 2016). Both parameters were calculated according to Equation 6 and 7.

$$LOD = \frac{3 * s_b}{a} \qquad (Eq.6)$$

$$LOQ = \frac{10 * s_b}{a} \qquad (Eq.7)$$

Where a is the slope of the calibration curve and s_b the standard deviation of the intercept.

Precision is a parameter that evaluates the degree of proximity between results, referred to the same sample. This parameter was obtained regarding repeatability (intra-day precision) (A. Alves, 2016). Repeatability is calculated by the standard deviation of, at least 6 determinations, of the same sample, at the same conditions. This parameter was calculated according to Equation 8.

$$CV\ \% = \frac{s * 100}{c} \qquad (Eq.8)$$

Where s is the standard deviation and c is the concentration (μg mL^{-1}).

Accuracy evaluates the degree of proximity between the obtained and expected results. It was measured by the recovery percentage (% R), that is calculated by the ratio between the expected result of concentration from the calibration curve and the obtained result (A. Alves, 2016).

The calibration curve of resveratrol was obtained by the linear relation between the absorbance values and the concentration values (μg mL^{-1}). Calibration curve is accepted as appropriate for analysis if it follows some parameters:

- It has to have at least 5 different standards solutions;
- The with linearity range factor must be greater than 10;
- Correlation coefficient (R) greater than 0.995;

Resveratrol's calibration curve was linear between 0.2 μg mL^{-1} and 6.1 μg mL^{-1} and the measurements were made in triplicate. The obtained calibration curve is presented in Figure 8.

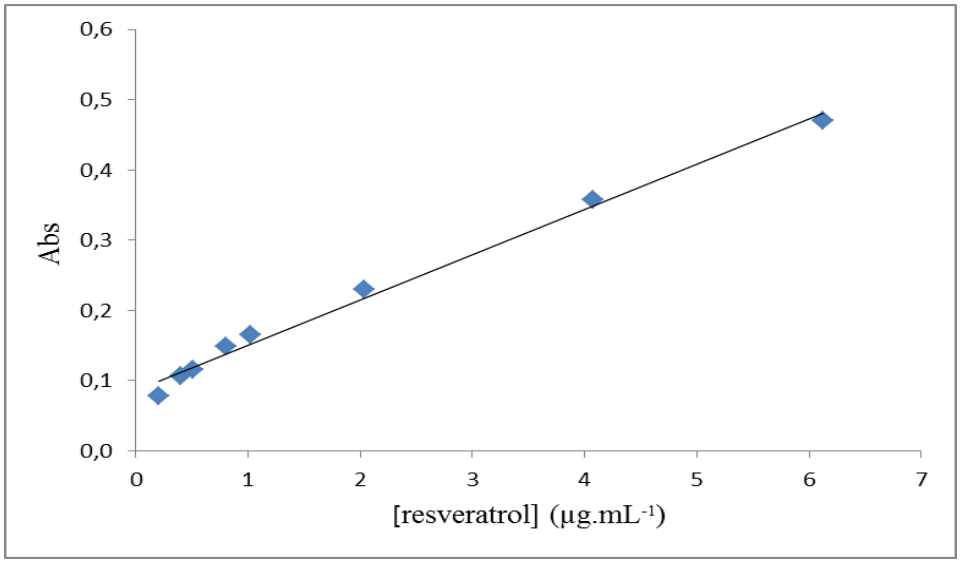

Figure 8 – Calibration curve of resveratrol in coconut oil.

As mentioned above, calibration curve is accepted as appropriate for analysis if it follows some parameters. Table 9 presents the values of the parameters obtained, as well as the LOD and LOQ.

Table 9 – Parameters and restrictions for the validation of calibration curve of resveratrol in coconut oil.

Parameter	**Restriction**	**Obtained Results**
Number of standards	≥ 5	8
Linearity range factor	≥ 10	31
Correlation coefficient	> 0.995	0.992
LOD (μg mL^{-1})	-	0.31
LOQ (μg mL^{-1})	-	1.0

Precision and accuracy parameters were also calculated and are presented in Table 10.

Table 10 – Repeatability and accuracy values obtained to the higher and middle concentration of standard solutions.

Standard (μg mL^{-1})	**Precision – Repeatability (% CV)**	**Accuracy (% R)**
6.10	2.3 %	97
2.04	5.3 %	109

In conclusion, it is possible to affirm that the method used is suitable for the quantification of resveratrol in coconut oil.